

SUB A3
We claim:

1. An *in vitro* immunoassay method for diagnosing human gastric intestinal metaplasia which comprises the steps of:

5 (a) contacting a gastric tissue sample of a subject suspected of having human gastric intestinal metaplasia cells with the monoclonal antibody DAS-1, or a fragment thereof, which monoclonal antibody is produced by the hybridoma deposited under ATCC accession number HB 9397 and which reacts with human gastric intestinal metaplasia antigen; and

10 (b) detecting immunoreactivity between the gastric tissue and the monoclonal antibody, such immunoreactivity indicating a positive diagnosis of human gastric intestinal metaplasia.

2. The method according to claim 1, wherein the human gastric intestinal metaplasia antigen is colon epithial specific protein.

3. The method according to claim 1, wherein the antibody or fragment is directly or indirectly attached to a detectable label.

20 4. The method according to claim 1, wherein detecting immunoreactivity is performed by immunoperoxidase staining, immunofluorescence, immunoelectronmicroscopy, or ELISA.

5. The method according to claim 4, wherein the immunoassay method is immunoperoxidase staining.

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6. The method according to claim 5, wherein the immunoperoxidase staining comprises:

- 30 (a) deparaffinizing the intestinal tissue by heating;
(b) immersing the deparaffinized tissue in xylene;
(c) rehydrating the tissue in decreasing concentrations of alcohol;
(d) washing the rehydrated tissue in neutral PBS;
(e) reducing the aldehydes of the washed tissue of step (d);
(f) reacting the tissue with normal goat serum, the monoclonal antibody,
35 biotinylated goat anti-mouse antibody and avidin-biotin-peroxidase complex;
(g) treating the reacted tissue with diaminobenzidine;
(h) washing the diaminobenzidine-treated tissue;
(i) staining the washed tissue of step (h) with hematoxylin, eosin or both;

and

(j) examining the stained tissue under a microscope to detect the presence of immunoreactivity.

5 7. The method according to claim 6, which further comprises the step of trypsinizing the intestinal tissue after reducing the aldehydes in the tissue but before reacting the tissue with the goat serum, monoclonal antibody, biotinylated goat anti-mouse antibody and avidin-biotin-peroxidase complex.

10 8. The method according to claim 6, wherein the decreasing concentrations of alcohol used for rehydration are 100%, 95%, 70%, and 50% alcohol.

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15 9. The method according to claim 1, further comprising the step of performing a negative control assay on a negative control sample to detect human gastric intestinal metaplasia cells present in the negative control sample and comparing results of the assay in (b) with the results of the negative control assay, wherein the presence of human gastric intestinal metaplasia cells in the assay in (b) above the presence of human gastric intestinal metaplasia cells in the negative control assay indicates a positive diagnosis of human gastric intestinal metaplasia.

20 10. The method according to claim 1, further comprising the step of performing a positive control assay on a positive control sample to detect human gastric intestinal metaplasia cells present in the positive control sample.

25 11. An *in vitro* immunoassay method for screening for human gastric intestinal metaplasia, thereby indicating a predisposition for gastric carcinoma, which comprises the steps of:

30 (a) contacting a gastric tissue sample of a subject suspected of having human gastric intestinal metaplasia cells with the monoclonal antibody DAS-1, or a fragment thereof, which monoclonal antibody is produced by the hybridoma deposited under ATCC accession number HB 9397 and which reacts with human gastric intestinal metaplasia antigen; and

35 (b) detecting immunoreactivity between the gastric tissue and the monoclonal antibody, such immunoreactivity indicating a positive diagnosis of human gastric intestinal metaplasia.

12. The method according to claim 11, wherein the human gastric intestinal metaplasia antigen is colon epithial specific protein.

13. The method according to claim 11, wherein the antibody or fragment is directly or indirectly attached to a detectable label.

5 14. The method according to claim 11, wherein detecting immunoreactivity is performed by immunoperoxidase staining, immunofluorescence, immunoelectronmicroscopy, or ELISA.

30h 10 15. The method according to claim 11, wherein the immunoassay method is immunoperoxidase staining.

16. The method according to claim 15, wherein the immunoperoxidase staining comprises:

- 15 (a) deparaffinizing the intestinal tissue by heating;
(b) immersing the deparaffinized tissue in xylene;
(c) rehydrating the tissue in decreasing concentrations of alcohol;
(d) washing the rehydrated tissue in neutral PBS;
(e) reducing the aldehydes of the washed tissue of step (d);
(f) reacting the tissue with normal goat serum, the monoclonal antibody, biotinylated goat anti-mouse antibody and avidin-biotin-peroxidase complex;
20 (g) treating the reacted tissue with diaminobenzidine;
(h) washing the diaminobenzidine-treated tissue;
(i) staining the washed tissue of step (h) with hematoxylin, eosin or both;
and
(j) examining the stained tissue under a microscope to detect the presence of
25 immunoreactivity.

30 17. The method according to claim 16, which further comprises the step of trypsinizing the intestinal tissue after reducing the aldehydes in the tissue but before reacting the tissue with the goat serum, monoclonal antibody, biotinylated goat anti-mouse antibody and avidin-biotin-peroxidase complex.

35 18. The method according to claim 16, wherein the decreasing concentrations of alcohol used for rehydration are 100%, 95%, 70%, and 50% alcohol.

19. The method according to claim 16, further comprising the step of performing a negative control assay on a negative control sample to detect human gastric intestinal metaplasia cells present in the negative control sample and comparing results of the assay in (b) with the results of the negative control assay,

wherein the presence of human gastric intestinal metaplasia cells in the assay in (b) above the presence of human gastric intestinal metaplasia cells in the negative control assay indicates a positive diagnosis of human gastric intestinal metaplasia.

5 20. The method according to claim 16, further comprising the step of performing a positive control assay on a positive control sample to detect human gastric intestinal metaplasia cells present in the positive control sample.

10 21. An *in vivo* immunoassay method for diagnosing human gastric intestinal metaplasia which comprises the steps of:

15 (a) administering to a human, suspected of having human gastric intestinal metaplasia, the monoclonal antibody DAS-1, or a fragment thereof, which monoclonal antibody is produced by the hybridoma deposited under ATCC accession number HB 9397 and which reacts with human gastric intestinal metaplasia antigen and is tagged with an isotope; and

20 (b) detecting immunoreactivity between the human gastric intestinal metaplasia cells and the monoclonal antibody by external scanning, such immunoreactivity indicating a positive diagnosis of human gastric intestinal metaplasia.

25 22. The method according to claim 21, wherein the monoclonal antibody DAS-1 is administered to the human intravenously.

30 23. The method according to claim 21, wherein the human gastric intestinal metaplasia antigen is colon epithelial specific protein.

35 24. The method according to claim 21, wherein the monoclonal antibody DAS-1 is tagged with a radioisotope and immunoreactivity is detected by Immunoscintigraphy.

 25. The method according to claim 24, wherein the radioactive isotope is ^{99}Tc .

 26. The method according to claim 21, wherein the monoclonal antibody DAS-1 is tagged with a stable isotope and immunoreactivity is detected by magnetic resonance imaging.

 27. The method according to claim 26, wherein the stable isotope is selected from the group consisting of ^2H , ^{13}C , ^{15}N , and ^{19}F .

28. The method according to claim 21, further comprising the step of performing a negative control assay on a negative control sample to detect human gastric intestinal metaplasia cells present in the negative control sample and comparing results of the assay in (b) with the results of the negative control assay, wherein the presence of human gastric intestinal metaplasia cells in the assay in (b) above the presence of human gastric intestinal metaplasia cells in the negative control assay indicates a positive diagnosis of human gastric intestinal metaplasia.

29. The method according to claim 21, further comprising the step of performing a positive control assay on a positive control sample to detect human gastric intestinal metaplasia cells present in the positive control sample.

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